# A New TIPE of Phosphoinositide Regulator in Cancer

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**Specific phosphoinositide lipids promote cell growth and cancer. In this issue of *Cancer Cell*, Fayngerts and colleagues demonstrate that the TIPE3 protein enhances PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$, is overexpressed in certain cancers, and promotes tumorigenesis. TIPE3 can act as a lipid transfer protein and may constitute a novel phosphoinositide metabolism regulator.**

Phosphoinositides are a minor component of lipid membranes, yet they play key roles in many cellular functions from vesicle trafficking and motility to cell survival, with disruption of phospholipid homeostasis being linked to various human diseases (Balla, 2013; Billcliff and Lowe, 2014). In particular, deregulation of PtdIns(3,4,5)$P_3$ and, to a lesser extent, PtdIns(4,5)$P_2$ production has been clearly shown to promote cell survival and tumor progression. In fact, activating mutations in the lipid kinase *PIK3CA*, which phosphorylates PtdIns(4,5)$P_2$ to generate PtdIns(3,4,5)$P_3$ and loss-of-function in the PtdIns(3,4,5)$P_3$ phosphatase PTEN, are among the most common genetic changes found in cancer.

PtdIns(3,4,5)$P_3$ recruits pleckstrin homology (PH) domain-containing proteins, such as PDK1 and AKT, to the plasma membrane. Subsequent activation of AKT leads to phosphorylation of numerous substrates, including proteins involved in protein synthesis (TSC2, PRAS40), survival (FOXO, MDM2), and cell cycle progression (GSK3, p21) (Manning and Cantley, 2007).

PtdIns(4,5)$P_2$ is much more abundant than the transiently produced PtdIns(3,4,5)$P_3$ and can bind a large number of proteins by interacting with PH or FERM domains or polybasic stretches among others. PtdIns(4,5)$P_2$ is the precursor for PtdIns(3,4,5)$P_3$ production. In addition, it is cleaved by phospholipase C (PLC) into inositol-triphosphate and diacylglycerol, leading to calcium release and activation of protein kinase C family members, both of which have numerous and often context-dependent effects (Bunney and Katan, 2010). In addition, PtdIns(4,5)$P_2$ plays important roles in endocytosis and cytoskeletal organization through the effects of PtdIns(4,5)$P_2$-binding proteins.

Simplistically, proteins involved in phosphoinositide signaling can be divided into three categories. (1) Phosphoinositide-modifying enzymes: these include lipid kinases (e.g., PI3Ks), lipid phosphatases (PTEN, SHIP), and phospholipases (e.g., PLCs), which generate new lipid species and initiate and terminate signaling cascades. (2) Lipid binding proteins: proteins that contain lipid binding domains such as PH, FERM, and PX domains or poly-basic motifs can act as effectors downstream of different lipid species. In this issue of *Cancer Cell*, Fayngerts et al. (2014) have characterized a protein, TIPE3, which acts as a transfer protein for PtdIns(4,5)$P_2$ and falls into a third category: (3) proteins that transfer, present, and protect lipids.

TIPE3 is a previously uncharacterized member of the tumor necrosis factor-α-induced protein 8 (TNFAIP8 or TIPE) family of proteins that are involved in tumorigenesis and inflammation (Lou and Liu, 2011). Fayngerts et al. (2014) show that TIPE3 may also promote tumorigenesis through elevation of PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$ levels. They use immunohistochemistry to demonstrate that TIPE3 expression is upregulated in human cancers, including lung and esophageal cancers. Consistently, TIPE3 overexpression promotes cell and tumor growth in an NIH 3T3-HRas$^{V12}$ mouse xenograft model, while TIPE3 knockout mice exhibit delayed carcinogen-induced tumorigenesis. Combined with data from soft agar assays, cell cycle profiles, and cap-dependent translation, TIPE3 clearly promotes cell growth and cell survival. This is in contrast to other members of the TNFAIP8 family, which have been shown to decrease cell survival (Gus-Brautbar et al., 2012; Lou and Liu, 2011). Fayngerts et al. (2014) hypothesize that this difference in function may be accounted for by a unique N-terminal region present in TIPE3. Indeed, deletion of this region converts TIPE3 from a pro-survival to a pro-death protein, while fusion of the N-terminal region to TIPE2 inhibits its pro-death function.

Fayngerts et al. (2014) observe that ectopic expression of TIPE3 enhanced activating phosphorylations on AKT and MAPK, while knockdown had the opposite effect. Interestingly, this is dependent on PI3K and PLC activity, suggesting a role for TIPE3 in enhancing cellular PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$ levels. Consistent with this hypothesis, TIPE3 overexpression leads to enhanced levels of both lipids, measured by lipid overlay assays and confocal imaging. That this is a direct effect of TIPE3 is suggested by the fact that forced nuclear localization of TIPE3 increased nuclear PtdIns(4,5)$P_2$ levels.

In order to understand how TIPE3 was enhancing phosphoinositide levels, the crystal structure of TIPE3 was determined. Similar to other TNFAIP8 family members, TIPE3 contains a TH fold, which has a large hydrophobic cavity, suggesting the ability to bind lipophilic molecules. Indeed, by lipid overlay assay, TIPE3 (as well as TIPE1, TIPE2, and TNFAIP8) are able to bind a number of lipid species, most prominently PtdIns(4,5)$P_2$, PtdIns(3,5)$P_2$, PtdIns(3,4)$P_2$, and PtdIns(3,4,5)$P_3$. Importantly, this is dependent on the TH domain, because mutation within the hydrophobic cavity disrupts this binding as well as the ability of TIPE3 to promote PtdIns(3,4,5)$P_3$ levels and AKT activation.

TIPE3 thus has the ability both to bind and enhance phosphoinositide levels. There is no evidence that TIPE3 is a lipid-generating enzyme, suggesting that

the TH domain may either protect the lipids from being turned over or enhance production of certain lipid species. Fayngerts et al. (2014) explore this latter possibility and demonstrate that, in vitro, TIPE3 can bind to vesicles containing PtdIns(4,5)$P_2$ and intriguingly can act as a lipid transfer protein. TIPE3 is able to both extract PtdIns(4,5)$P_2$ from vesicles as well as insert solubilized PtdIns(4,5)$P_2$ into vesicles. Therefore, TIPE3 (and possibly the rest of the TNFAIP8 family) may represent a new class of phospholipid transfer proteins.

Phospholipid transfer proteins mediate the movement of phospholipids (including phosphatidylcholine, phosphatidylinositol, and sterols) between membranes and function as lipid sensors or presenting proteins (Wirtz, 2006). Phosphatidylinositol transfer proteins have been previously shown to have essential roles in organism survival, and, through intracellular transfer of the precursor molecule, PI, can have knock-on effects on the production of other lipid species including PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$ (Chang et al., 2013; Wirtz, 2006). However, to our knowledge, TIPE3 is the first transfer protein described that binds and transfers PtdIns(4,5)$P_2$. It will be important to determine if the function of other TNFAIP8 family members is at least partially dependent on their ability to bind phospholipids.

Mechanistically, this study opens up many questions about how exactly TIPE3 enhances lipid production. The authors most thoroughly address the role of TIPE3 in binding and regulating PtdIns(4,5)$P_2$. However TIPE3 was shown to bind a wider range of phospholipids, and it will be important to address the effect of TIPE3 on the metabolism of these lipid species. In addition, the exact function of TIPE3 in lipid metabolism is unclear. Because the major source of PtdIns(4,5)$P_2$ is within the plasma membrane, is TIPE3 changing the local concentrations of PtdIns(4,5)$P_2$? Or is the major function of TIPE3 to transfer a precursor phospholipid from elsewhere in the cell to the plasma membrane, where it can then be converted to PtdIns(4,5)$P_2$?

Alternatively, is the major physiological function of TIPE3 to act as a lipid presenting protein? This is currently a source of debate in the field of lipid transfer proteins and has proved a difficult question to answer in vivo. Fayngerts et al. (2014) provide some evidence that TIPE3 can function as a lipid-presenting protein; as in an in vitro lipid kinase assay, TIPE3 enhances PtdIns(3,4,5)$P_3$ production by PI3K.

Taken together, the identification of TIPE3 introduces us to a new class of lipid regulators that can directly regulate the levels of PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$. Future work will be important to assess the exact mechanism of action, not only from a lipid biochemistry perspective, but also in biology, signaling, and disease.

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## REFERENCES

Balla, T. (2013). Physiol. Rev. *93*, 1019–1137.

Billcliff, P.G., and Lowe, M. (2014). Biochem. J. *461*, 159–175.

Bunney, T.D., and Katan, M. (2010). Nat. Rev. Cancer *10*, 342–352.

Chang, C.L., Hsieh, T.S., Yang, T.T., Rothberg, K.G., Azizoglu, D.B., Volk, E., Liao, J.C., and Liou, J. (2013). Cell Reports *5*, 813–825.

Fayngerts, S.A., Wu, J., Oxley, C.L., Liu, X., Vourekas, A., Cathopoulis, T., Wang, Z., Cui, J., Liu, S., Sun, H., et al. (2014). Cancer Cell *26*, this issue, 465–478.

Gus-Brautbar, Y., Johnson, D., Zhang, L., Sun, H., Wang, P., Zhang, S., Zhang, L., and Chen, Y.H. (2012). Mol. Cell *45*, 610–618.

Lou, Y., and Liu, S. (2011). Mol. Immunol. *49*, 4–7.

Manning, B.D., and Cantley, L.C. (2007). Cell *129*, 1261–1274.

Wirtz, K.W. (2006). FEBS Lett. *580*, 5436–5441.